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14. ABSTRACT This basic research initiative has been an ongoing interdisciplinary effort to lay the foundation for developing, novel effective and safe non-lethal technologies that alter skeletal muscle contraction and/or neural functioning (i.e., neurosecretion) via radiofrequency (RF)/microwave (MW) electromagnetic radiation. Major accomplishments included 1) completion of studies examining the effect of 1 to 6 GHz MW fields on catecholamine release from chromaffin cells and 2) completion of studies on the effect of 0.75 to 1 GHz RF fields on skeletal muscle contraction, using in each study fixed frequencies and frequency sweep paradigms. A newer research effort aimed at examining the effects of nanosecond electric pulses of high intensity on neurosecretory chromaffin cells and elucidating the mechanism of interaction has been expanded, with very promising and exciting results being generated. Over the course of the funding period, the research was presented at four international meetings and has resulted in one doctoral dissertation, two peer-reviewed papers, one manuscript under review, and two manuscripts that are close to being submitted.					
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## **FINAL PERFORMANCE REPORT**

**Technical Proposal entitled:** “Directed Energy Non-Lethal Weapons”

**Award Number:** FA9550-07-1-0592

**Start Date:** 30 September 2007

**Termination Date:** 29 March 2010

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## **ABSTRACT**

This basic research initiative has been an ongoing interdisciplinary effort to lay the foundation for developing novel, effective and safe non-lethal technologies that alter skeletal muscle contraction and/or neural functioning (i.e., neurosecretion) via radiofrequency (RF)/microwave (MW) electromagnetic radiation. Major accomplishments included 1) completion of studies examining the effect of 1 to 6 GHz MW fields on catecholamine release from chromaffin cells and 2) completion of studies on the effect of 0.75 to 1 GHz RF fields on skeletal muscle contraction, using in each study fixed frequencies and frequency sweep paradigms. A second research effort aimed at examining the effects of nanosecond electric pulses of high intensity on neurosecretory chromaffin cells and elucidating the mechanism of interaction has been expanded, with very promising and exciting results being generated. Over the course of the funding period, the research was presented at four international meetings and has resulted in one doctoral dissertation, two peer-reviewed papers, one manuscript under review, and two manuscripts that are close to being submitted.

## **EXECUTIVE SUMMARY**

### **Objectives**

The research in this proposal was a transition from AFOSR FA9550-06-1-0377 that was aimed at sustaining the progress and growth of on-going research projects in which non-thermal radiofrequency /microwave (RF/MW) effects on skeletal muscle contraction and catecholamine release from chromaffin cells were being investigated. Another objective from AFOSR FA9550-06-1-0377 that also transitioned into this grant was to develop further the capability to explore effects of nanosecond duration, high intensity electric field effects on chromaffin cells, with this research effort continuing as a collaboration with AFOSR-funded researchers at the University of Southern California (USC) who were one of two groups instrumental in developing nanoelectropulse technology.

### **Accomplishments/New Findings**

- 1) Effect of MW fields (1 to 6 GHz frequency range) on catecholamine release: studies were completed with apparent non-thermal effects on CA release noted most often with delivery of 5-6 GHz MW fields applied as a frequency sweep. These novel findings will set the stage for further investigations into potential applications of MW fields on neural-type cells that involve non-thermal mechanisms.
- 2) Effect of RF fields (0.75 to 1 GHz frequency range) on skeletal muscle force production: even after a variety of exposure parameters and protocols were tried, we did not observe robust and reproducible non-thermal effects on contractile force due to the RF fields applied. However, because in these studies as well as to those alluded to in 1) above a great deal of attention was given to the design and characterization of the exposure setups, the research overall has significant value with respect to advancing further investigations into the potential for RF/MW bioeffects induced via a non-thermal mechanism.

- 3) Effect of high intensity nanosecond electric pulses on chromaffin cells: with nanosecond pulsers and engineering expertise provided by colleagues at USC, we have observed that with the application of only a single 5 nsec, 5 MV/m electric pulse there is a pronounced, transient influx of calcium into the cells that is sufficient in magnitude to stimulate catecholamine release without any discernible adverse effects on the cells. Investigations to understand the mechanism of interaction of the high intensity electric field with the cells will give us clues as to how this novel type of stimulus can be best applied for potential military and medical applications. This project will continue via the successful procurement of funding from other sources (see below).
- 4) As an overall accomplishment, the interdisciplinary nature of all of the projects has provided invaluable basic research training opportunities for undergraduate, graduate, and post-graduate students.

## **Publications**

Vernier, P.T., Sun, Y., Chen, M.-T., Gundersen, M.A., and Craviso, G.L. (2008). Nanosecond electric pulse-induced calcium entry into chromaffin cells. *Bioelectrochemistry* 73:1-4.

Craviso, G. L., Chatterjee, P., Maalouf, G., Cerjanic, A., Yoon, J., Chatterjee, I., and Vernier, P. T. Nanosecond electric pulse-induced increase in intracellular calcium in adrenal chromaffin cells triggers calcium-dependent catecholamine release. *IEEE Trans. Dielectr. Electr. Insul.* 16:1294-1301, 2009.

Craviso, G.L. Choe, S., Chatterjee, P., Chatterjee, I. and Vernier, P.T., Nanosecond Electric Pulses: a Novel Stimulus for Triggering  $\text{Ca}^{2+}$  Influx into Chromaffin Cells via Voltage-gated  $\text{Ca}^{2+}$  Channels. Submitted to *Cell. Molec. Neurobiol.*

Craviso, G. L., Wiese R., Hagan, T., McPherson, D. and Chatterjee, I. Effect of 750 – 1000 MHz Radiofrequency/Microwave Fields On Skeletal Muscle Force Development. In Preparation.

Yoon, J., McPherson, D., Chatterjee, I. and Craviso, G.L., Non-thermal effects of pulsed microwave fields on catecholamine release from chromaffin cells. In Preparation.

## **Personnel Supported**

Gale L. Craviso, Ph.D., Professor of Pharmacology – Principal Investigator

Indira Chatterjee, Ph.D., Professor of Electrical and Biomedical Engineering – Co-Principal Investigator

Dana McPherson, Associate Engineer, Dept. of Electrical and Biomedical Engineering

Weihua Guan, Ph.D., post-doctoral Fellow

Jihwan Yoon, Ph.D. in Electrical Engineering, December 2008

Paroma Chatterjee, graduate student (until 31 August 2009)

Robert Wiese, research assistant

Sophie Choe, research assistant (part-time)

Horace Goff, research assistant (full and part-time)

Karla Bee, graduate research assistant (part-time)  
Tim Lindgren, graduate student; laboratory technician (part-time)  
Marc Cerruti, undergraduate lab aide (part-time); research assistant (part-time)  
Alex Cerjanic, laboratory technician (part-time)  
Camron Wipfli, undergraduate lab aide (part-time)

## **Interactions/Transitions**

### **a) *Presentations***

#### **Oral:**

Craviso, G. L., Maalouf, G., Choe, S., Chen, M. T., McPherson, D., Chatterjee, I., Gundersen, M. A. and Vernier, P. T., Nanosecond electric pulse stimulates catecholamine release from chromaffin cells. 30<sup>th</sup> Annual Bioelectromagnetics Society Meeting, San Diego, CA, June 2008.

Chatterjee, P., Vernier, P. T., Chatterjee, I. and Craviso, G. L., Single nanosecond electric pulse-induced influx of calcium into adrenal chromaffin cells requires extracellular sodium. 31<sup>st</sup> Annual Bioelectromagnetics Society Meeting, Davos, Switzerland, 2009.

Craviso, G. L., Chatterjee, P., Chatterjee, I. and Vernier, P.T., Ca<sup>2+</sup>-dependent nanosecond excitation of catecholamine release. 15<sup>th</sup> International Symposium on Chromaffin Cell Biology, Merida, Mexico, 2009 (Invited speaker)

#### **Poster:**

Guan, W., Hagan, T., Chatterjee, I., McPherson, D. and Craviso, G. L., Narrowband and broadband radiofrequency/microwave exposure system for real-time monitoring of cellular responses. 30<sup>th</sup> Annual Bioelectromagnetics Society Meeting, San Diego, CA, June 2008.

Craviso, G. L., Maalouf, G., Choe, S. McPherson, D. Chatterjee, I., Gundersen, M. A. and Vernier, P. T., Nanosecond electric pulse-induced catecholamine release from chromaffin cells. Gordon Conference on Bioelectrochemistry, Biddeford, ME, July 2008.

Yoon, J., Chatterjee, I., McPherson, D. and Craviso, G. L., Microwave fields cause changes in catecholamine release from chromaffin cells. 31<sup>st</sup> Annual Bioelectromagnetics Society Meeting, 2009.

Cerjanic, A. Chatterjee, I., McPherson, D. and Craviso, G. L., Design and fabrication of a perfusion microelectrode chamber for high intensity electric field stimulation using rapid prototyping techniques. 31<sup>st</sup> Annual Bioelectromagnetics Society Meeting, 2009.

#### **Other:**

Jihwan Yoon obtained his Ph.D. in Electrical Engineering in December 2008; his dissertation is entitled "Non-thermal effects of pulsed microwave fields on catecholamine release from

chromaffin cells: exposure system design and characterization, and experimental data”. The dissertation can be accessed as follows:

1. Go to <http://www.knowledgecenter.unr.edu/>
2. In quick search box, enter “Yoon” and select author as search criteria and click “Go”
3. In next list, select “Yoon Jihwan 1972”
4. On the next page, under the title and at the top of the blue box it says “Click the following to view” – select “abstract”
5. On the next page, to the right is a box labeled “Other available formats.” Click “Full Text - PDF.”

The research was featured in the June 2008 issue of Defense Technology International

One of our radiofrequency exposure setups was featured in a review article: Lovisolo, G.A., Apollonio, F., Ardoino, L., Liberti, M., Lopresto, V., Marino, C., Paffi, A. and Pinto, R., Specifications of *in vitro* exposure setups in the radiofrequency range. Radio Science Bulletin 331, page 26, 2009.

**b) Consultative and advisory functions** – None

**c) Transitions** – Craviso, Chatterjee and Vernier, as Co-PIs, have already applied for funding from the National Institutes of Health to continue the research on nanoelectropulse exposure of chromaffin cells.

**New Discoveries, Inventions or patent disclosures:** None

**Honors/Awards** - None

## COMPREHENSIVE TECHNICAL SUMMARY

### Rationale

Working toward the development of novel technologies that are based on electromagnetic fields (in particular RF/MW and high intensity pulsed electric fields) and are capable of affecting human functioning by causing alterations in neural (neurosecretory) activity and skeletal muscle contraction has been ongoing research effort. This effort has been driven by the potential for using the information derived from these studies in the design of non-lethal weapons that target these physiological processes in a safe and effective manner. For our studies, special emphasis was given to studying non-thermal RF/MW effects on two *in vitro* biological systems, neurosecretory adrenal chromaffin cells that synthesize, store and release catecholamines, and skeletal muscle that is responsible for voluntary movement. To work further toward developing effective applications of pulsed, high intensity RF/MW electromagnetic fields, we have also been collaborating with P. Thomas Vernier at USC, an effort we felt would greatly accelerate the goal of coming up with practical outcomes for our research.

## **Specific Projects and Outcomes**

### ***1) Identifying non-thermal RF/MW effects (1 to 6 GHz frequency range) on catecholamine release: Project completed – manuscript being prepared for submission to Bioelectromagnetics.***

The free space exposure setup for exposing chromaffin cells to MW fields in the 1 to 6 GHz frequency range (published in Yoon et al., 2006) was modified to achieve optimal performance for conducting well-controlled biological experiments. Briefly, the exposure system consisted of a cell perfusion apparatus (CPA) inside which chromaffin cells were immobilized on a glass fiber filter (GFF). The cells were continuously superfused at a rate of 1.0 ml/min with a balanced salt solution (BSS) maintained at  $36.5 \pm 0.2^{\circ}\text{C}$ . The temperature of the BSS entering and exiting the CPA was continuously monitored in the inlet and outlet tubing with non-perturbing fluoroptic temperature probes placed as close as physically possible to the GFF where the cells are immobilized. The CPA was mounted vertically within a mini anechoic chamber and the cells exposed to MW fields in the frequency range 1 - 6 GHz by positioning the CPA in the near field of a high power broadband horn antenna (Figure 1, Appendix). Catecholamine release was monitored by an electrochemical detector placed in-line with the CPA outlet tubing (Figure 2, Appendix).

A series of studies were carried out delivering continuous wave and pulsed MW fields at either fixed frequencies or using the novel exposure paradigm of pulsed frequency sweeps (PFS; each sweep spanning one GHz). Of several PFS exposure experiments carried out in the 1 to 6 GHz frequency range using different pulse parameters, a one GHz frequency sweep spanning 5 to 6 GHz using a pulse width of 100 ms (Figure 3, Appendix) elicited significant effects on catecholamine release most often (Table 1, Appendix). The entire body of work served as partial fulfillment of the requirement for a doctoral degree awarded to Jihwan Yoon and is near completion for submission to Bioelectromagnetics.

### ***2. RF/MW exposure setup for real-time monitoring of effects on chromaffin cells via fluorescence microscopy: project on-hold.***

With prior funding a novel exposure system that can deliver high electric field RF/MW pulse modulated radiation, broadband Gaussian pulses or RF/MW modulated Gaussian pulses with frequency spectrum centered in the band 0.75 – 6 GHz had been fabricated for use with an inverted microscope for real-time fluorescence imaging of effects on chromaffin cells, such as changes in intracellular calcium (Hagan et al., 2006). A photograph of the setup is presented in Figure 4 (Appendix). Briefly, chromaffin cells are immobilized on collagen-coated indium tin oxide (ITO) cover slips, which comprise the bottom of a specially designed circular cell perfusion chamber. Cells are loaded with the fluorescent calcium indicator dye, Calcium Green-1 (Figure 4, Appendix) and the cell chamber attached to the exposure device in which RF/MW fields are delivered to the cells by means of a carefully designed coaxial applicator having a highly tapered inner conductor. The entire setup is mounted on the stage of an epifluorescence microscope, and images of the cells are captured before, during and after exposure. Cells are continuously perfused throughout an experiment with BSS maintained at  $36.5 \pm 0.2^{\circ}\text{C}$  and a drug

stimulus (nicotinic receptor agonist) injected at varying intervals for assessing effects on both basal intracellular calcium level and receptor-mediated increases in intracellular calcium level (Guan et al., 2008). We had planned to expand these efforts by also *1*) exploring non-thermal RF/MW induced changes in membrane potential, *2*) predicting the spatial distribution and time evolution of the electric field and specific absorption rate (SAR) inside of and surrounding both single chromaffin cells and cells in aggregates, using the Finite-Difference Time-Domain (FDTD) technique to help reveal where the electric fields couple with the greatest amount of energy to intracellular structures and to specific regions of the chromaffin cell membrane where ion channels and receptors are located, and *3*) adapting the exposure system for also studying non-thermal RF/MW effects on skeletal muscle, using single skeletal muscle fibers to monitor intracellular calcium via fluorescence imaging. However, these efforts got only a brief start due to two problems. First, the coaxial applicator got corroded due to a barely detectable and continuous leakage of the BSS onto the coaxial applicator during experiments. While this problem was eventually resolved, another issue arose, namely that both of the individuals carrying out these studies had to leave the institution during the funding period for personal reasons. The project was consequently placed on hold, with our objective being to obtain additional funding at a future date that will allow us to recruit personnel with the appropriate background and experience and to carry these projects forward.

***3) Identifying non-thermal RF/MW effects on skeletal muscle contraction: Project completed – manuscript being prepared for submission to Bioelectromagnetics.***

Experiments using a waveguide-based setup for exposing a fast-twitch skeletal muscle, specifically *flexor digitorum brevis*, a toe muscle obtained from mice, to 0.75 to GHz fields (setup described in Lambrecht et al., 2006 and shown in Figure 5, Appendix) are now completed, with no significant or reproducible non-thermal effects on contractile force observed when using a variety of exposure parameters and conditions. A manuscript that is very near completion will be submitted to Bioelectromagnetics.

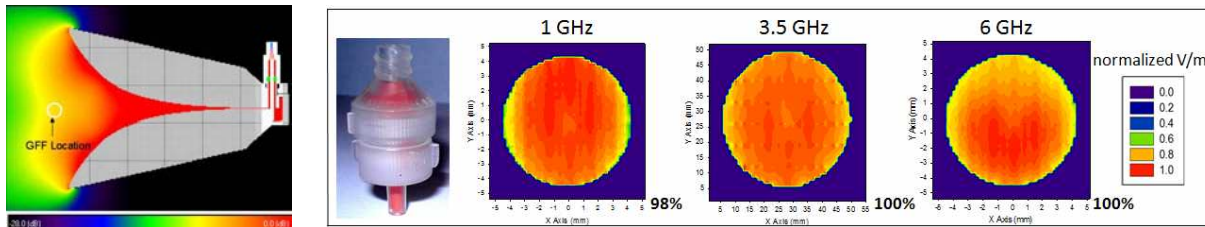
***4) Effect of nanosecond pulses on chromaffin cells: Project planned to be on-going.***

Studies assessing the effects of nanosecond duration, high intensity electric pulses on chromaffin cells, a project that was an objective in grant FA9550-06-1-0377 and continued in this grant, constitute a very successful collaboration with P. Thomas Vernier at USC. We first reported that a single 4-5 nsec, 5 MV/m electric pulse stimulates calcium influx into chromaffin cells via L-type voltage-gated calcium channels (Vernier et al., 2008) and that this single burst of calcium influx is sufficient in magnitude to be of physiological significance, that is, to result in the release of catecholamine (Craviso et al., 2009). Using the fluorescence microscopy - nanosecond pulse exposure setup shown in Figure 6 (Appendix) we have since determined that multiple types of voltage-gated calcium channels are activated (Table 2, Appendix) in a sodium-dependent manner (Figure 7, Appendix). Interestingly, our results so far raise the possibility that the pulse causes an influx of sodium through lipid nanopores rather than through endogenous protein ion channels, suggesting a novel way by which the cells get depolarized. These most recent findings are reported in a manuscript under review (Craviso et al., submitted).

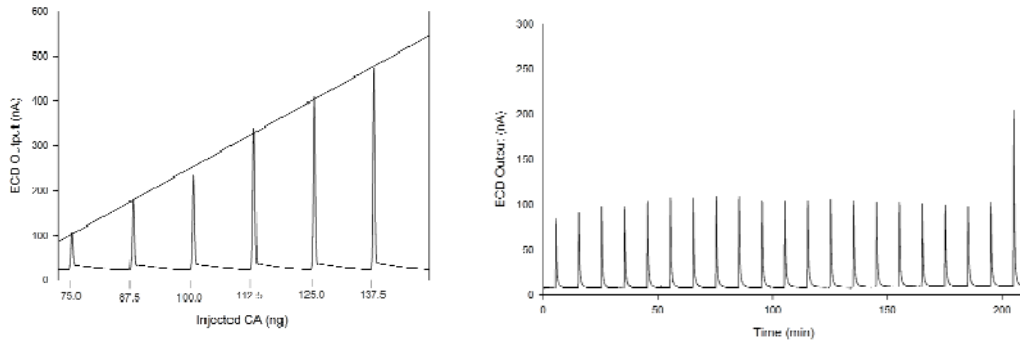
While we await the outcome of the review of a pending grant application with the National Institutes of Health that would sustain this project over the next five years, funds available from another AFOSR grant are enabling us to keep these studies on-going until the end of December 2010.

## APPENDIX

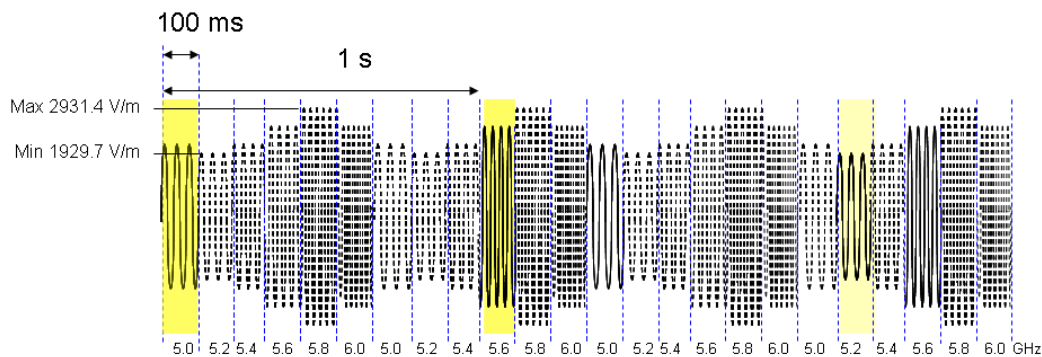
### *1) Identifying non-thermal RF/MW effects (1 to 6 GHz frequency range) on catecholamine release.*



**Figure 1.** (Left) Computed near field distribution of the broadband horn antenna at the plane of the GFF at 3.5 GHz. (Right) Photograph of the CPA (left) and the E-field distributions (right) on the GFF placed in the near field at 1, 3.5 and 6 GHz. Each field distribution is normalized to the maximum value. % refers to the area of the GFF homogeneous to within 30%.



**Figure 2.** Verification of the linearity and reproducibility of the response of the ECD for monitoring catecholamine release during MW exposure of the cells. (Left) The amount of the catecholamine standard injected was increased from 75 to 137.5 ng by increasing the injection volume from 10 to 18.35  $\mu$ l, while maintaining an injection duration of 10 s for all injections. (Right) Ten  $\mu$ l injections containing 75 ng of the catecholamine standard were delivered every 10 min for up to four hours, for a total of 20 injections. The mean value was  $96.9 \pm 12.4$  nA.

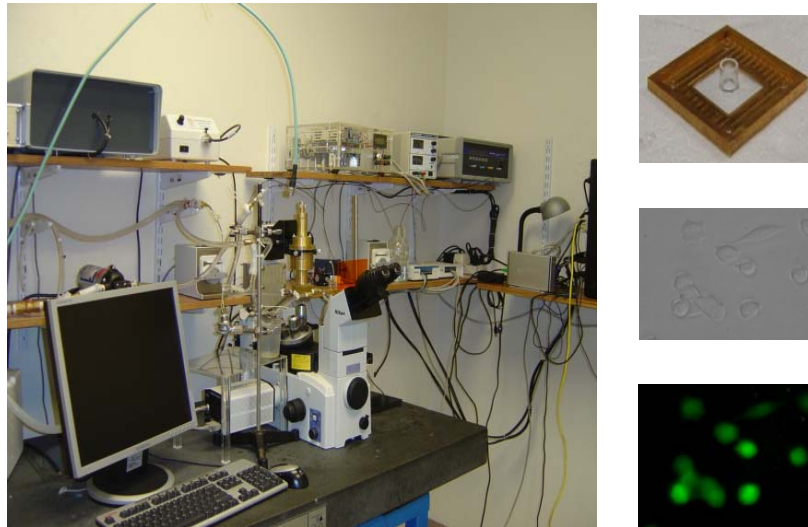


**Figure 3.** Illustration of the 5-6 GHz PFS with a sweep time of 600 ms, a pulse width of 100 ms and a repetition rate of 1 Hz at the location of the cells. The magnitudes of the wave at different frequencies vary since the gain of the amplifier, the gain of the broadband horn antenna and the loss of the power cable vary at different frequencies. The magnitudes were found using XFDTD results and the measured cable loss.

**Table 1.** Summary of results for exposures of varying pulse widths for 5-6 GHz PFS.

5 - 6 GHz PFS		
Pulse Width		
10 ns	Number of exposures	7
	Number of exposures affecting CA release	4
	% of exposures affecting CA release	57%
	Number of CA peaks affected	10
	% difference from the trend line (mean $\pm$ S.D.)	9.96 $\pm$ 3.60%
100 ms	Number of exposures	8
	Number of exposures affecting CA release	6
	% of exposures affecting CA release	75%
	Number of CA peaks affected	30
	% difference from the trend line (mean $\pm$ S.D.)	17.92 $\pm$ 13.50 %
100 $\mu$ s	Number of exposures	7
	Number of exposures affecting CA release	2
	% of exposures affecting CA release	29%
	Number of CA peaks affected	6
	% difference from the trend line (mean $\pm$ S.D.)	13.33 $\pm$ 3.69 %

***2. RF/MW exposure setup for real-time monitoring of effects on chromaffin cells via fluorescence microscopy.***



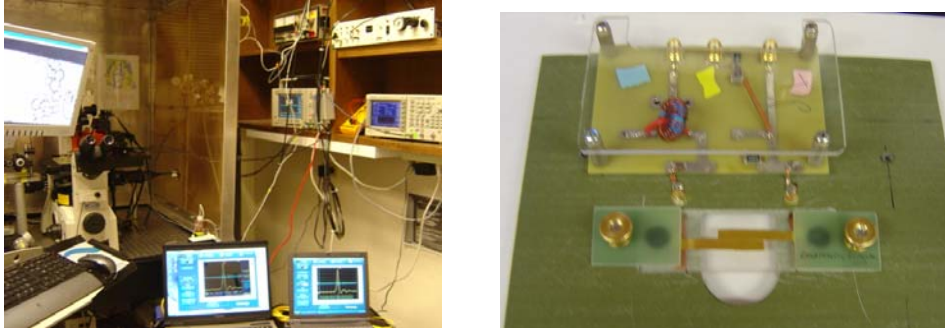
**Figure 4.** Photograph of the exposure device mounted on an inverted microscope (left), with a photograph of the cell chamber shown (top right). Bright field (middle right) and corresponding fluorescent field (bottom right) micrographs of chromaffin cells attached to the bottom of the cell chamber and loaded with the calcium indicator dye Calcium Green-1.

***3) Identifying non-thermal RF/MW effects on skeletal muscle contraction.***



**Figure 5.** Photograph of the waveguide-based exposure system that was used for assessing RF effects on skeletal muscle contraction

#### 4) Effect of nanosecond pulses on chromaffin cells.

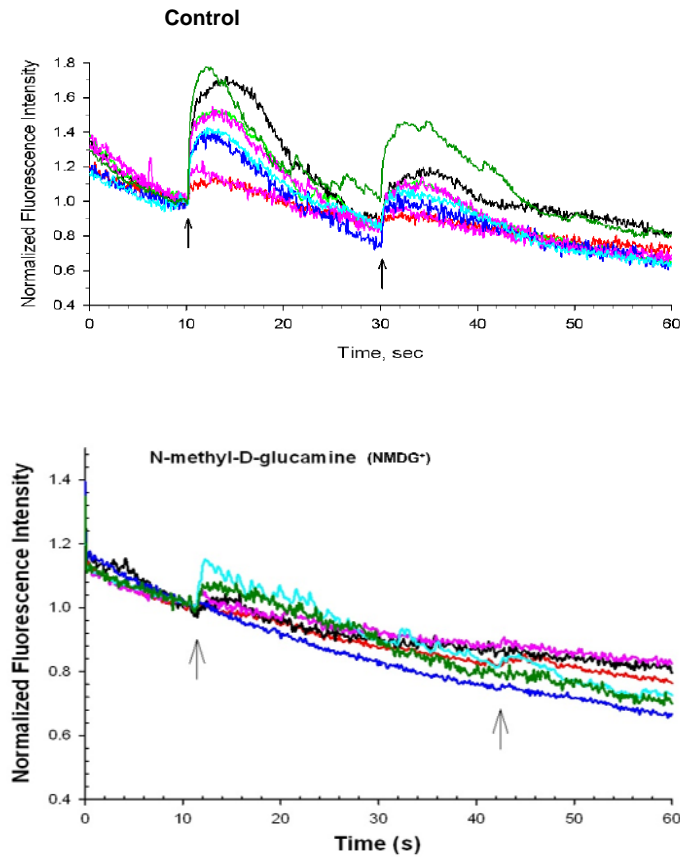


**Figure 6.** Photograph of the setup for observing effects of nanosecond pulses via fluorescence imaging (left). Exposures are carried out at room temperature within a microelectrode chamber (photograph at right) mounted on the stage of a Nikon TE 2000 epifluorescence microscope, and intracellular calcium level monitored in real-time in cells loaded with the calcium indicator dye, Calcium Green-1.

**Table 2.** Effect of blocking different types of voltage-gated calcium channels (VGCC) on the nanoelectropulse-induced increase in intracellular calcium level.

VGCC Inhibitors	Total Number of Cells	Cells Responding (%*)	Fluorescence Intensity (% of control responses*)
<b>L-type channels</b>			
None	127	87 (79-91)	—
Nimodipine (5 $\mu$ M)	84	54 (35-73)	49 (37-61)
<b>N-type channels</b>			
None	312	86 (74-95)	—
$\omega$ -Conotoxin GVIA (10 nM)	180	67 (35-100)	70 (35-100)
$\omega$ -Conotoxin GVIA (20 nM)	95	58 (36-74)	59 (53-67)
<b>P/Q-type channels</b>			
None	471	84 (82-91)	—
$\omega$ -Agatoxin IVA (30 nM)	54	74	91
$\omega$ -Agatoxin IVA (100 nM)	183	54 (44-71)	50 (29-75)
$\omega$ -Agatoxin IVA (300 nM)	100	29 (11-50)	48 (46-50)
<b>N- and P/Q-type channels</b>			
None	126	97 (95-98)	—
$\omega$ -Conotoxin MVIIC (300 nM)	149	72 (58-86)	64 (42-87)

\*Average value from two or more experiments, with the range in parentheses



**Figure 7.** Effect of removing extracellular sodium on the ability of a nanosecond pulse to induce a rise in intracellular calcium. (Top) Control response. (Bottom) Response when sodium is replaced with N-methyl-D-glucamine. Fluorescence intensity is normalized to point at which the first nanoelectropulse is applied.

## References

- Craviso, G. L., Chatterjee, P., Maalouf, G., Cerjanic, A., Yoon, J., Chatterjee, I., and Vernier, P. T. Nanosecond electric pulse-induced increase in intracellular calcium in adrenal chromaffin cells triggers calcium-dependent catecholamine release. *IEEE Trans. Dielectr. Electri. Insul.* 16:1294-1301, 2009.
- Craviso, G.L. Choe, S., Chatterjee, P., Chatterjee, I. and Vernier, P.T. Nanosecond Electric Pulses: a Novel Stimulus for Triggering  $\text{Ca}^{2+}$  Influx into Chromaffin Cells via Voltage-gated  $\text{Ca}^{2+}$  Channels. Submitted to *Cell. Molec. Neuobiol.*

Guan, W., Hagan, T., Chatterjee, I., McPherson, D. and *Craviso, G. L.*, Narrowband and broadband radiofrequency/microwave exposure system for real-time monitoring of cellular responses. 30<sup>th</sup> Annual Bioelectromagnetics Society Meeting, 2008.

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Lambrecht, M.R., Chatterjee, I., McPherson, D., Quinn, J., Hagan, T. and Craviso, G.L. Design, characterization and optimization of a waveguide-based RF/MW exposure system for studying nonthermal effects on skeletal muscle contraction. IEEE Trans. Plasma Sci. 34:1470-1479, 2006.

Vernier, P.T., Sun, Y., Chen, M.-T. Gundersen, M.A. and Craviso, G.L., Nanosecond electric pulse-induced calcium entry into chromaffin cells. Bioelectrochemistry 73:1-4, 2008.

Yoon, J., Chatterjee, I., McPherson, D. and Craviso, G.L. Design, characterization and optimization of a broadband mini exposure chamber for studying catecholamine release from chromaffin cells exposed to microwave radiation: Finite-Difference Time-Domain Technique. IEEE Trans. Plasma Sci. 34:1455-1469, 2006.